FEBS 30113 FEBS Letters 579 (2005) 6423–6427

Antibiotic susceptibility of mammalian mitochondrial translation

Li Zhang[a,c], Ng Ching Ging[b], Taeko Komoda[a], Takao Hanada[b,1], Tsutomu Suzuki[a,b,*], Kimitsuna Watanabe[a,*]

[a] *Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan*
[b] *Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan*
[c] *State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, PR China*

Received 25 July 2005; accepted 22 September 2005

Available online 2 November 2005

Edited by Lev Kisselev

Abstract All medically useful antibiotics should have the potential to distinguish between target microbes (bacteria) and host cells. Although many antibiotics that target bacterial protein synthesis show little effect on the translation machinery of the eukaryotic cytoplasm, it is unclear whether these antibiotics target or not the mitochondrial translation machinery. We employed an in vitro translation system from bovine mitochondria, which consists of mitochondrial ribosomes and mitochondrial elongation factors, to estimate the effect of antibiotics on mitichondrial protein synthesis. Tetracycline and thiostrepton showed similar inhibitory effects on both *Escherichia coli* and mitochondrial protein synthesis. The mitochondrial system was more resistant to tiamulin, macrolides, virginiamycin, fusidic acid and kirromycin than the *E. coli* system. The present results, taken together with atomic structure of the ribosome, may provide useful information for the rational design of new antibiotics having less adverse effects in humans and animals.

Keywords: Protein synthesis; Antibiotics; Ribosome; Mitochondria; *E. coli*

1. Introduction

Protein synthesis is a multi-step process that includes initiation, elongation, termination and recycling. Each process requires ribosomes, tRNAs and several translation factors. The ribosome is a universally conserved ribonucleoprotein complex that catalyzes the ordered polymerization of amino acids directed by the genetic information on mRNA. Various clinically useful antibiotics prevent protein synthesis by interacting with the bacterial ribosome or translation factors. Good antibiotics do not target the cytoplasmic ribosomes of host cells because their structures are adapted to fit the structures of bacterial ribosomes and not those of eukaryotic ribosomes. Mitochondrial (mt) ribosomes are categorized as a bacteria-type ribosome on the basis of shared antibiotic susceptibilities and sequence similarities of ribosomal proteins and RNAs [7,11,22,31–33]. If antibiotics could penetrate or be transported into mitochondria, the mt ribosome would be exposed to the antibiotics, resulting in the inhibition of mt translation. Aminoglycosides, which target bacterial ribosomes to induce misreading, are the most commonly used antibiotics. Autotoxicity is a major irreversible adverse effect of aminoglycosides and occurs in a dose-dependent fashion [13]. The A1555G mutation in mt 12S rRNA gene confers susceptibility to aminoglycosides and can cause non-syndromic deafness [13,28]. Although the molecular pathogenesis of aminoglycoside-induced deafness is not fully understood, it is possible that aminoglycosides directly bind to the mt rRNA and induce translation disorders in protein synthesis [15].

Using a modified 'fragment reaction' (puromycin reaction) for measuring the peptidyl-transferase activity of the ribosomal large subunit, the antibiotic susceptibility of the bovine mt 39S large subunit was compared to those of *Escherichia coli* and bovine cytoplasmic ribosomal subunits many years ago [11]. The mt large subunit showed low susceptibility to lincosamines and macrolides, indicating that the binding sites for certain of these antibiotics had been altered.

With the advent of an in vitro mt translation system established by our group from bovine mitochondria [16,23,34], it is now possible to characterize several antibiotics with respect to their abilities to target the small subunit as well as the large subunit of the mt ribosome. In addition, kirromycin and fusidic acid, which target elongation factors, can also be characterized in this system. The present study describes the antibiotic susceptibility of the mammalian mt translation system.

2. Materials and methods

2.1. Materials

E. coli total tRNA was purchased from Boehringer Mannheim. Tetracycline, fusidic acid, thiostrepton, kirromycin and virginiamycin M1 were purchased from Sigma–Aldrich. Josamycin, spiramycin, midecamycin acetate were gifts from Meiji seika Co. Ltd. Tiamulin was a gift from Novartis Co. Ltd, Shanghai, PR China. [^{14}C] phenylalanine was purchased from Amersham-Pharmacia.

2.2. Preparation of ribosome, elongation factors and aminoacyl-tRNA

The 55S mt ribosome and 70S *E. coli* ribosome were prepared as described previously [16]. Mt EF-G was partially purified by DEAE–Sepharose and Superdex 200 chromotography (Amersham-Pharmacia) from bovine liver [9]. The concentration of mt EF-G was determined by quantifying the intensity of the CBB-stained band on a SDS–PAGE gel by using Fluor-S MAX (Bio-rad). Recombinant *E. coli* EF-Tu,

*Corresponding authors. Present address: Biological Information Research Center, National Institute of Advanced Science and Technology, 2-42 Aomi, Koto-ku, Tokyo 135-0064, Japan.
E-mail addresses: ts@chembio.t.u-tokyo.ac.jp (T. Suzuki), kwatanab@jbirc.aist.go.jp (K. Watanabe).

[1] Present address: Division of Molecular Cell Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan.

0014-5793/$30.00
doi:10.1016/j.febslet.2005.09.103

E. coli EF-G, and bovine mt EF-Tu were expressed in *E. coli* and purified as described [30,40]. The aminoacylation of *E. coli* tRNA was carried out with an *E. coli* S100 fraction under the conditions described previously [23], and protein synthesis was quantified by measuring the amount of incorporated [^{14}C] phenylalanine using a scintillation counter (ALOKA, Liquid Scintillation Counter, LSC-6100).

2.3. Measurement of the inhibition of poly(U)-directed poly(Phe) synthesis by antibiotics

The in vitro translation was carried out as described in the literature [16,34] with slight modifications. Appropriate amounts of antibiotics and 0.08 μM *E. coli* ribosomes were pre-incubated for 25 min on ice, and then at 37 °C for 5 min in a buffer containing 50 mM Tris–HCl (pH 7.5), 6.5 mM $MgCl_2$ and 60 mM KCl. Poly(U)-directed polyphenylalanine synthesis by *E. coli* ribosomes was performed in 20 μL of the same buffer containing 0.08 μM *E. coli* ribosomes (pre-incubated with each antibiotic), 0.5 mM GTP, 2 mM DTT, 0.1 mM spermine, 1 mg/ml poly U, 25 mM phosphoenolpyruvate, 2.5 unit/ml pyruvate kinase, 0.3 μM [^{14}C] Phe-tRNA, 0.6 μM EF-G, 0.6 μM EF-Tu, and each antibiotic at 37 °C for 20 min. Then, 10 μL of the reaction mixture was spotted onto a filter paper (Whattman 3MM), followed by deacylation of aminoacyl-tRNA through boiling. The TCA-insoluble products were then quantified by liquid scintillation counting (ALOKA, LSC-6100).

For the mt translation system, the pH of the Tris–HCl buffer was changed to 8.5, and the concentrations of $MgCl_2$, KCl, DTT and Spermine were changed to 7.5, 5, 1, and 0.5 mM, respectively. All the other procedures, except for ribosomes, EF-G and EF-Tu, which were all mitochondrial, were the same as those used in *E. coli* translation system. The initial experiments were performed over a wide range of antibiotic concentrations (0.1 μM to 10 mM) to obtain a rough estimation of the IC_{50} value for each antibiotic. Based on the results, we determined the IC_{50} value for each antibiotic within a narrow rage of concentrations both in *E. coli* and mt systems. Each experiment was repeated at least three times, and the average was taken to be the final IC_{50} value.

2.4. Site-directed mutagenesis of E. coli ribosomal 23S rRNA

We used *E. coli* strain NT101 (derived from TA542 [2] which was kindly provided by Dr. Cathy Squires of Tuft University) which is deleted for all chromosomal rRNA operons and, instead, contains a rescue plasmid, pRB101, carrying the *rrnB* and *sacB* genes. Using a Quick change site-directed mutagenesis kit (Stratagene), the A2058G point mutation was introduced into the 23S rRNA gene on another plasmid, pRB102, which shares the same replication origin (pSC101) as pRB101 but has a different antibiotic marker (Km). NT101 cells were transformed with the A2058G mutant plasmid, and colonies resistant to kanamycin and sucrose were selected to obtain a strain having a ribosome with the A2058G mutation.

3. Results and discussion

The poly(U)-directed polyphenylalanine synthesis system of mitochondria was constructed with bovine mt ribosomes, recombinant mt EF-Tu and partially purified mt EF-G from bovine liver mitochondria. The conditions were optimized as described previously [16,23,34]. As a control system, we employed the *E. coli* system which consists of *E. coli* ribosomes, recombinant *E. coli* EF-Tu, and EF-G. Polyphenylalanine incorporation for 20 min. was plotted against the concentration of each antibiotic and the IC_{50} value of each antibiotic was then added to Table 1.

Tiamulin, a pleuromulin derivative, is known to strongly inhibit bacterial translation in vitro and the peptidyl transferase (PTase) reaction between formylmethionyl (fMet)-tRNA and puromycin [12,20,27]. The IC_{50} of tiamulin in the *E. coli* system was determined to be 0.48 μM, while the IC_{50} in the mt system was 58.1 μM (Fig. 1A and Table 1), which represents nearly a one hundred-fold difference. Tiamulin occupies the PTase center, and thereby sterically hinders the correct positioning of tRNA [5,27]. The binding sites of tiamulin at the PTase center of *E. coli* 23S rRNA have been assigned as A2058, A2059, U2506, U2584 and U2585 (according to the *E. coli* numbering system) by antibiotic foot-printing [27]. A comparison of the tiamulin binding sites in *E. coli* and mt rRNAs, shows that A2058 is replaced by a G in mitochondria, while all the other binding sites are conserved. Therefore, the resistance of the mt system to tiamulin is probably conferred by the base difference at position 2058 in the mt large subunit rRNA. To confirm this, we constructed an *E. coli* ribosome with the A2058G mutation by site-directed mutagenesis and measured its IC_{50} value with tiamulin. As expected, the IC_{50} of the A2058G *E. coli* ribosome increased to 18.2 μM. This strongly indicates that the resistance of the mt system to tiamulin is due to the presence of a G residue in the mt rRNA binding site.

Macrolide antibiotics are powerful inhibitors of protein synthesis in bacteria [14]. These antibiotics are composed of a large lactone ring (from 14 to 16 carbon atoms) to which several sugars (sometimes amino sugars) are attached. They can be broadly divided structurally and functionally into relatively homogenous groups, as represented by the erythromycin and spiramycin groups [8]. The spiramycin group carries a 16-membered lactone ring, of which only one position, C5, is frequently glycosylated with a disaccharide. Macrolide specifically binds to the PTase center and to the entrance of the peptide tunnel in the 50S subunit. It interferes with the interaction of peptidyl-tRNA with the ribosomal P-site, and/or induces a conformational change in the ribosome [4,17,18,29,35].

In the present study, we investigated the susceptibility of *E. coli* and mt ribosomes to three 16-membered macrolides, spiramycin, josamycin and midecamycin. The results (Fig. 1B and Table 1) clearly indicated that IC_{50} values for these macrolides are much higher in the mt system than in the *E. coli* system. The main binding sites for these macrolides are certain bases located within the central loop of domain V of 23S

Table 1
IC_{50} values (μM) of various antibiotics in *E. coli* and mitochondrial systems

Antibiotics	Mitochondria	*E. coli*	*E. coli* A2058G	Categories	Target
Tiamulin	58.1 ± 2.6	0.48 ± 0.04	18.2 ± 2.3	Pleuromutilin	Large subunit
Josamycin	12.3 ± 1.6	0.35 ± 0.04	59.5 ± 2.5	Macrolide	Large subunit
Spiramycin	24.9 ± 0.5	0.27 ± 0.04	37.2 ± 0.63	Macrolide	Large subunit
Midecamycin	76.6 ± 1.6	0.43 ± 0.03	78.2 ± 4.6	Macrolide	Large subunit
Virginiamycin	3.5 ± 0.2	0.22 ± 0.02	–	Mikamycin	Large subunit
Kirromycin	64.9 ± 2.3	0.30 ± 0.02	–		EF-Tu
Fusidic acid	2020 ± 90	33.0 ± 3.0	–		EF-G
Thiostrepton	0.60 ± 0.05	0.48 ± 0.02	–		Large subunit
Tetracycline	170 ± 10	150 ± 10	–		Small subunit

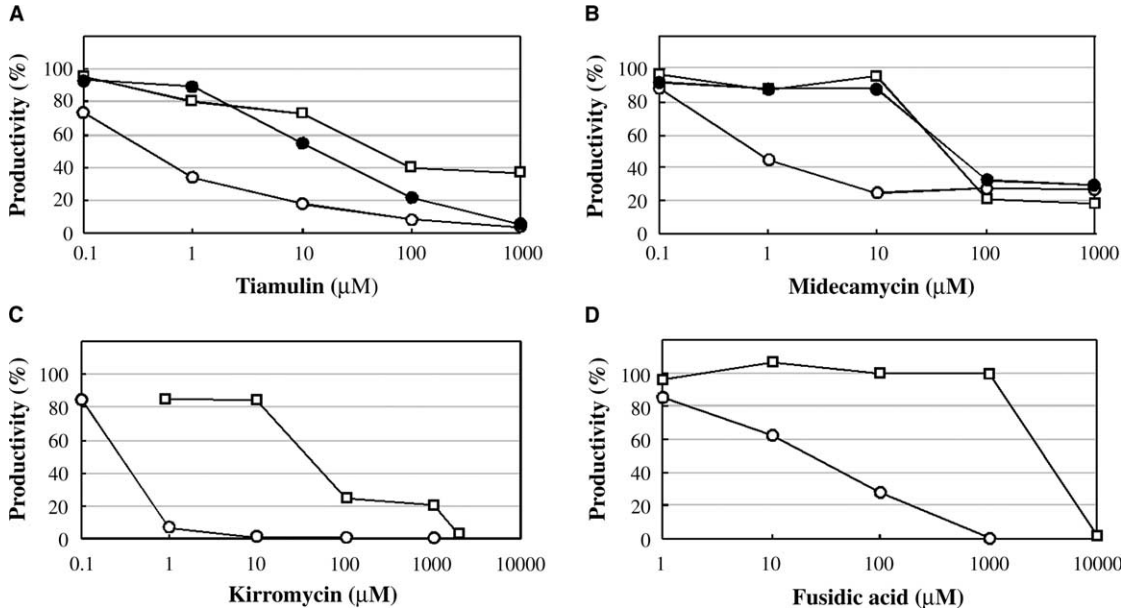

Fig. 1. Inhibition of protein synthesis in mt and *E. coli* translation systems by antibiotic titration. Inhibition curves for tiamulin (A), midecamycin (B), kirromycin (C) and fusidic acid (D) are shown. Mitochondria (open square), *E. coli* wild-type (open circle) and *E. coli* with the A2058G mutant ribosome (closed circle).

rRNA, such as G2505, A2058, U2609 and A2062 (according to the *E. coli* numbering system) [8,17,29]. As in the case of tiamulin, A2058 is known to be one of the most important sites for macrolide binding. We confirmed that a A2058G mutation in the *E. coli* ribosome increases resistance to macrolides by 2 orders of magnitude (Table 1 and Fig. 1B). Taken together with previous studies [11], our data demonstrate that the resistance of mt ribosomes to macrolides results from a single base replacement at position 2058 in mt ribosomal RNA.

Virginiamycin occupies both the A and P sites, and thus blocks two steps in the peptide chain elongation process[18]. This antibiotic also simultaneously induces a conformational change in the ribosome nearby the PTase center [18]. In our system, the IC_{50} values of virginiamycin M1 in the *E. coli* and mt systems was determined to be 0.22 and 3.5 μM, respectively. The results suggest that the mt system has slightly greater resistance to virginiamycin M1 compared to the *E. coli* system. Since the binding site of this antibiotic is known to overlap with that of macrolides [18], resistance to virginiamycin M1 in the mt system might be explained by a similar mechanism that reduces affinity for both macrolide and virginiamycin M1 binding.

The elongation factors, EF-G and EF-Tu, interact consecutively with the ribosome during polypeptide synthesis, and their functions depend on GTP binding and hydrolysis. After GTP hydrolysis, GDP-bound EF-Tu and EF-G dissociate from the ribosome. Kirromycin and fusidic acid are antibiotics that target EF-Tu and EF-G, respectively. Neither kirromycin nor fusidic acid inhibit GTP hydrolysis by either factor, but prevent the dissociation of both factors from the ribosome after GTP hydrolysis [19,24].

Kirromycin inhibits the release of EF-Tu from the ribosome and freezes the EF-Tu · GDP and aminoacyl-tRNA complex on the ribosome [37,38]. In this study, the IC_{50} values of kirromycin in the *E. coli* and mt systems were determined to be 0.30 and 64.9 μM, respectively (Fig. 1C and Table 1). The more than 200-fold higher value in the mt system suggests that mt EF-Tu is resistant to kirromycin. In *E. coli* EF-Tu, several amino acid residues, such as L120, Q124, Y160, G316, Q329, A375 and E378, have been reported to be responsible for kirromycin resistance [1,24,25]. A comparison of the amino acid sequences of mt and *E. coli* EF-Tu revealed that the resistance of mt translation to kirromycin might be due to F160, V329, T375 and D378 (according to the *E. coli* numbering system) in mt EF-Tu. In fact, it has been reported that *E. coli* EF-Tu with the A375T mutation has increased resistance to kirromycin as measured by poly U-directed poly(Phe) synthesis [24].

Fusidic acid, a steroid antibiotic, blocks EF-G · GDP on the ribosome after peptidyl-tRNA translocation and thereby further protein synthesis [19]. In our experiment, the IC_{50} for fusidic acid in mitochondria and *E. coli* were determined to be 2.02 mM and 33.0 μM, respectively (Fig. 1D). Thus, mt EF-G displays higher resistance to fusidic acid. Several mutations in bacterial EF-G that confer resistant to fusidic acid are located close to the interface between domains G and 5 of EF-G [19,21]. It was reported that the IC_{50} of fusidic acid in *E. coli* was increased significantly when Ala at position 138 in domain G was replaced with Thr [21], which is identical to the amino acid at the corresponding position in mt EF-G.

Thiostrepton binds tightly to the GTPase-associated region of 23S rRNA and inhibits ribosome-associated GTPase events [3,36,39]. Similar IC_{50} values for thiostrepton were observed in *E. coli* (0.48 μM) and mt (0.60 μM) systems. A1067 is the main binding site for thiostrepton in this region. In fact, A1067 is also conserved in mt rRNA.

Tetracycline binds primarily to the 30S ribosome subunit and blocks the binding of aminoacyl-tRNA to the A site of this subunit [6,10,26]. The primary binding site is located in the head of the 30S subunit but another sites are also located in the body. In mt ribosomes, the bases that form the primary binding site are slightly different from those of *E. coli*. However, both *E. coli* and mt ribosomes had similar IC_{50} values

for tetracycline. This may be explained by the fact that the phosphate backbone of rRNA is the main interaction site for tetracycline. It is known that tetracycline is a group of antibiotics against a wide range of Gram-positive and Gram-negative bacteria. In addition, bacterial resistance to tetracycline is usually not acquired by mutations or modifications in rRNA. Similar susceptibility to tetracycline found in mt ribosome can be explained by the unique character of this antibiotic.

It is also important to measure ribosomal susceptibility to different aminoglycosides, which bind the decoding center of the small subunit to induce misreading. However, the current in vitro translation cannot be used to test these antibiotic, since it is impossible to detect misreading by using a homopolymeric RNA template. Development of an in vitro mt translation system with non-homopolymeric mRNAs or natural mRNAs should enable us to resovle this issue.

4. Conclusion

We have measured the antibiotic susceptibility of the mt ribosome and mt elongation factors by measuring IC_{50} values for several antibiotics. Although tetracycline and thiostrepton efficiently targeted the mt ribosome, tiamulin, three macrolides and virginiamycin did not. In addition, mt EF-Tu and mt EF-G were shown to be resistant to kirromycin and fusidic acid, respectively. Thus, the mt translation system is substantially resistant to most but not all antibiotics. Understanding the molecular basis of these differences in antibiotic susceptibility is expected to provide a framework for the development of new antibiotics for the next generation.

Acknowledgments: We thank all the former and present laboratory member at the University of Tokyo, and especially, Drs. Neuza S. Sato, Maki Tarasaki and Yohei Kirino whose contributions were greatly appreciated. We also thank Dr. Linda L. Spremulli (University of North Carolina) for providing us with the mt EF-Tu expression vector and Dr. Cathy Squires (Tufts University) for the *E. coli* strain. L.Z. is a Chinese researcher supported by the Ministry of Education of China and the Ministry of Education, Science, Sports, and Culture of Japan. This work was supported by grants-in-aid for scientific research on priority areas from the Ministry of Education, Science, Sports, and Culture of Japan (to K.W. and T.S.) and by a grant from the Human Frontier Science Program RGY23/2003 (to T.S.).

References

[1] Abdulkarim, F., Liljas, L. and Hughes, D. (1994) Mutations to kirromycin resistance occur in the interface of domains I and III of EF-Tu · GTP. FEBS Lett. 352, 118–122.
[2] Asai, T., Zaporojets, D., Squires, C. and Squires, C.L. (1999) An *Escherichia coli* strain with all chromosomal rRNA operons inactivated: complete exchange of rRNA genes between bacteria. Proc. Natl. Acad. Sci. USA 96, 1971–1976.
[3] Bausch, S.L., Poliakova, E. and Draper, D.E. (2005) Interactions of the N-terminal domain of ribosomal protein L11 with thiostrepton and rRNA. J. Biol. Chem. 22, 22.
[4] Berisio, R., Schluenzen, F., Harms, J., Bashan, A., Auerbach, T., Baram, D. and Yonath, A. (2003) Structural insight into the role of the ribosomal tunnel in cellular regulation. Nat. Struct. Biol. 10, 366–370.
[5] Bosling, J., Poulsen, S.M., Vester, B. and Long, K.S. (2003) Resistance to the peptidyl transferase inhibitor tiamulin caused by mutation of ribosomal protein l3. Antimicrob. Agents Chemother. 47, 2892–2896.
[6] Brodersen, D.E., Clemons Jr., W.M., Carter, A.P., Morgan-Warren, R.J., Wimberly, B.T. and Ramakrishnan, V. (2000) The structural basis for the action of the antibiotics tetracycline, pactamycin, and hygromycin B on the 30S ribosomal subunit. Cell 103, 1143–1154.
[7] Cavdar Koc, E., Burkhart, W., Blackburn, K., Moseley, A. and Spremulli, L.L. (2001) The small subunit of the mammalian mitochondrial ribosome. Identification of the full complement of ribosomal proteins present. J. Biol. Chem. 276, 19363–19374.
[8] Champney, W.S. and Tober, C.L. (2000) Specific inhibition of 50S ribosomal subunit formation in Staphylococcus aureus cells by 16-membered macrolide, lincosamide, and streptogramin B antibiotics. Curr. Microbiol. 41, 126–135.
[9] Chung, H.K. and Spremulli, L.L. (1990) Purification and characterization of elongation factor G from bovine liver mitochondria. J. Biol. Chem. 265, 21000–21004.
[10] Connell, S.R., Tracz, D.M., Nierhaus, K.H. and Taylor, D.E. (2003) Ribosomal protection proteins and their mechanism of tetracycline resistance. Antimicrob. Agents Chemother. 47, 3675–3681.
[11] Denslow, N.D. and O'Brien, T.W. (1978) Antibiotic susceptibility of the peptidyl transferase locus of bovine mitochondrial ribosomes. Eur. J. Biochem. 91, 441–448.
[12] Dornhelm, P. and Hogenauer, G. (1978) The effects of tiamulin, a semisynthetic pleuromutilin derivative, on bacterial polypeptide chain initiation. Eur. J. Biochem. 91, 465–473.
[13] Fischel-Ghodsian, N. (1999) Genetic factors in aminoglycoside toxicity. Ann. NY Acad. Sci. 884, 99–109.
[14] Goldman, R.C. and Scaglione, F. (2004) The macrolide–bacterium interaction and its biological basis. Curr. Drug Targets Infect. Disord. 4, 241–260.
[15] Hamasaki, K. and Rando, R.R. (1997) Specific binding of aminoglycosides to a human rRNA construct based on a DNA polymorphism which causes aminoglycoside-induced deafness. Biochemistry 36, 12323–12328.
[16] Hanada, T., Suzuki, T., Yokogawa, T., Takemoto-Hori, C., Sprinzl, M. and Watanabe, K. (2001) Translation ability of mitochondrial tRNAsSer with unusual secondary structures in an in vitro translation system of bovine mitochondria. Genes Cells 6, 1019–1030.
[17] Hansen, J.L., Ippolito, J.A., Ban, N., Nissen, P., Moore, P.B. and Steitz, T.A. (2002) The structures of four macrolide antibiotics bound to the large ribosomal subunit. Mol. Cell 10, 117–128.
[18] Hansen, J.L., Moore, P.B. and Steitz, T.A. (2003) Structures of five antibiotics bound at the peptidyl transferase center of the large ribosomal subunit. J. Mol. Biol. 330, 1061–1075.
[19] Hansson, S., Singh, R., Gudkov, A.T., Liljas, A. and Logan, D.T. (2005) Structural insights into fusidic acid resistance and sensitivity in EF-G. J. Mol. Biol. 348, 939–949.
[20] Hodgin, L.A. and Hogenauer, G. (1974) The mode of action of pleuromutilin derivatives. Effect on cell-free polypeptide synthesis. Eur. J. Biochem. 47, 527–533.
[21] Johanson, U. and Hughes, D. (1994) Fusidic acid-resistant mutants define three regions in elongation factor G of *Salmonella typhimurium*. Gene 143, 55–59.
[22] Koc, E.C., Burkhart, W., Blackburn, K., Moyer, M.B., Schlatzer, D.M., Moseley, A. and Spremulli, L.L. (2001) The large subunit of the mammalian mitochondrial ribosome. Analysis of the complement of ribosomal proteins present. J. Biol. Chem. 276, 43958–43969.
[23] Kumazawa, Y., Schwartzbach, C.J., Liao, H.X., Mizumoto, K., Kaziro, Y., Miura, K., Watanabe, K. and Spremulli, L.L. (1991) Interactions of bovine mitochondrial phenylalanyl-tRNA with ribosomes and elongation factors from mitochondria and bacteria. Biochim. Biophys. Acta 1090, 167–172.
[24] Mesters, J.R., Zeef, L.A., Hilgenfeld, R., de Graaf, J.M., Kraal, B. and Bosch, L. (1994) The structural and functional basis for the kirromycin resistance of mutant EF-Tu species in *Escherichia coli*. EMBO J. 13, 4877–4885.
[25] Parmeggiani, A. and Swart, G.W. (1985) Mechanism of action of kirromycin-like antibiotics. Annu. Rev. Microbiol. 39, 557–577.
[26] Pioletti, M., Schlunzen, F., Harms, J., Zarivach, R., Gluhmann, M., Avila, H., Bashan, A., Bartels, H., Auerbach, T., Jacobi, C., Hartsch, T., Yonath, A. and Franceschi, F. (2001) Crystal structures of complexes of the small ribosomal subunit with tetracycline, edeine and IF3. EMBO J. 20, 1829–1839.

[27] Poulsen, S.M., Karlsson, M., Johansson, L.B. and Vester, B. (2001) The pleuromutilin drugs tiamulin and valnemulin bind to the RNA at the peptidyl transferase centre on the ribosome. Mol. Microbiol. 41, 1091–1099.
[28] Prezant, T.R., Agapian, J.V., Bohlman, M.C., Bu, X., Oztas, S., Qiu, W.Q., Arnos, K.S., Cortopassi, G.A., Jaber, L. and Rotter, J.I., et al. (1993) Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nat. Genet. 4, 289–294.
[29] Schlunzen, F., Zarivach, R., Harms, J., Bashan, A., Tocilj, A., Albrecht, R., Yonath, A. and Franceschi, F. (2001) Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature 413, 814–821.
[30] Shimizu, Y., Inoue, A., Tomari, Y., Suzuki, T., Yokogawa, T., Nishikawa, K. and Ueda, T. (2001) Cell-free translation reconstituted with purified components. Nat. Biotechnol. 19, 751–755.
[31] Spremulli, L.L., Coursey, A., Navratil, T. and Hunter, S.E. (2004) Initiation and elongation factors in mammalian mitochondrial protein biosynthesis. Prog. Nucleic Acid Res. Mol. Biol. 77, 211–261.
[32] Suzuki, T., Terasaki, M., Takemoto-Hori, C., Hanada, T., Ueda, T., Wada, A. and Watanabe, K. (2001) Proteomic analysis of the mammalian mitochondrial ribosome. Identification of protein components in the 28 S small subunit. J. Biol. Chem. 276, 33181–33195, Epub 2001 Jun 11.
[33] Suzuki, T., Terasaki, M., Takemoto-Hori, C., Hanada, T., Ueda, T., Wada, A. and Watanabe, K. (2001) Structural compensation for the deficit of rRNA with proteins in the mammalian mitochondrial ribosome. Systematic analysis of protein components of the large ribosomal subunit from mammalian mitochondria. J. Biol. Chem. 276, 21724–21736, Epub 2001 Feb 21.
[34] Takemoto, C., Koike, T., Yokogawa, T., Benkowski, L., Spremulli, L.L., Ueda, T.A., Nishikawa, K. and Watanabe, K. (1995) The ability of bovine mitochondrial transfer RNAMet to decode AUG and AUA codons. Biochimie 77, 104–108.
[35] Tu, D., Blaha, G., Moore, P.B. and Steitz, T.A. (2005) Structures of MLSBK antibiotics bound to mutated large ribosomal subunits provide a structural explanation for resistance. Cell 121, 257–270.
[36] Uchiumi, T., Wada, A. and Kominami, R. (1995) A base substitution within the GTPase-associated domain of mammalian 28 S ribosomal RNA causes high thiostrepton accessibility. J. Biol. Chem. 270, 29889–29893.
[37] Valle, M., Sengupta, J., Swami, N.K., Grassucci, R.A., Burkhardt, N., Nierhaus, K.H., Agrawal, R.K. and Frank, J. (2002) Cryo-EM reveals an active role for aminoacyl-tRNA in the accommodation process. EMBO J. 21, 3557–3567.
[38] Vogeley, L., Palm, G.J., Mesters, J.R. and Hilgenfeld, R. (2001) Conformational change of elongation factor Tu (EF-Tu) induced by antibiotic binding. Crystal structure of the complex between EF-Tu · GDP and aurodox. J. Biol. Chem. 276, 17149–17155, Epub 2001 Jan 30.
[39] Wimberly, B.T., Guymon, R., McCutcheon, J.P., White, S.W. and Ramakrishnan, V. (1999) A detailed view of a ribosomal active site: the structure of the L11–RNA complex. Cell 97, 491–502.
[40] Woriax, V.L., Burkhart, W. and Spremulli, L.L. (1995) Cloning, sequence analysis and expression of mammalian mitochondrial protein synthesis elongation factor Tu. Biochim. Biophys. Acta 1264, 347–356.